

EXTREME PLEOMORPHISM AND THE BACTERIAL LIFE CYCLE: A FORGOTTEN CONTROVERSEY

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The first 40 years of this century witnessed bacteriologists involved in a debate which was fought with an intensity not seen since the arguments over spontaneous generation conducted during the last quarter of the 19th century. This now long-forgotten controversy concerned the question of whether or not bacteria exhibit extreme pleomorphism and go through complex life cycles. The term *pleomorphism* was used to refer to the supposed ability of bacteria to change shape dramatically, or to exist in a number of extreme morphological forms. Thus it was believed that bacteria could change from a single coccoid to complex filamentous forms and vice versa. In addition, rather than reproducing by single division, bacteria were thought to undergo complex life cycles involving single cells, spore, filaments, and ultra-filterable forms.

The debate split microbiologists into two opposing schools: the monomorphists and the pleomorphists. The monomorphists finally triumphed, but as we shall see, even today reports continue to appear apparently showing that bacteria exhibit extreme morphological variations and undergo complex life cycles.

Nearly all modern microbiologists belong to the monomorphic school; that is, they accept that, apart from minor variation, each bacterial cell is derived from a previously existing cell of practically the same size and shape. Cocci generally beget cocci, and rods give rise to rods. The monomorphist view, stressed by Virchow, Cohn and Koch, is that by binary fission most bacteria divide transversely to produce two new cells which eventually achieve the same size and morphology of the original. In the same way, a single spore germinates to give rise to a vegetative cell essentially the same as the cell from which the spore originated. Exceptions to this rule are accepted in certain so-called higher bacteria, including some actinomycetes. Simple bacteria, on the other hand, are generally regarded as showing only occasional, slight morphological variation. This view of bacterial morphology and growth is so enshrined in our view of these organisms that we rarely bother to think about it. Despite this, there are a small number of latter-day heretics who continue to provide evidence which, they claim, supports the pleomorphist heresy.

The Historical Literature on Extreme Pleomorphism and the Bacterial Growth Cycle.

The original pleomorphists were particularly active during the first three decades of this century. The basic tenet of their belief was that even common bacteria showed complex life cycles which often included a frequently pathogenic, filterable, or hidden phase [1]. Some even suggested that bacteria are merely rudimentary components of the fungal life

cycle. The principal proponents of pleomorphism, such as Almquist, Bergstrand, Hort, Lohnis, Mellon, and Enderlein, have largely been forgotten. However, even renowned microbiologists like Ferdinand Cohn published evidence in support of extreme pleomorphism. Similarly, the eminent American bacteriologist, Theobald Smith, isolated a bacterium which apparently occurred in three forms: a bacillus, a coccus with an endospore or arthrospore, and a conglomeration of all three [2].

By 1928, in an article on morphology published in the monograph *The Newer Knowledge of Bacteriology and Immunology*, Clark could state that "bacteria, even amongst the Eubacteriales, do at times reproduce by means other than equal fission seems to me to be definitely proved" [4]. He quotes the work of Hort, who showed that under adverse conditions, colon-typhoid bacteria reproduce by budding, by producing Y-shaped and large aberrant forms and deeply staining granules which can be filterable [5, 6]. Hort went on to describe how these irregular bodies reproduced actively and so were not examples of so-called involution forms, a term used by the monomorphists to suggest that what the pleomorphists were seeing was merely a collection of freakish, unreproducible forms produced by old cells. These were invariably sterile, incapable of taking up a stain, and were produced in old cultures by localised cell-wall lysis. However, such unusual forms could also be seen in young cultures [5, 6]. Alexander Fleming also described how one of his four-day-old cultures of an anaerobic streptococcus changed from its usual chains of cocci to a variety of strain shapes which he regarded as being involution forms [7].

Lohnis concluded that all bacteria live alternatively in first an organised and then an amorphous state [3]. The latter he called the "symplastic state," because at this point the living matter enclosed in separate cells apparently undergoes a thorough mixing, followed by the complete disintegration of cell wall, to form a non-stainable symplasm. Lohnis also suggested that direct union between two or more cells may occur by the process which he termed "conjugation.". He also stated that all bacteria multiply not only by fission, but by the formation of gonidia. These were sometimes seen to grow directly into full-sized cells, or to go through a symplasm stage. Such gonidia were either produced by partial or complete dissolution of the cell wall or developed while still united to the mother cell. Some of the gonidia were also filterable. Lohnis' main conclusion was that the life cycle of each bacterial species comprises several sub-cycles showing wide morphological and physiological variations, all being connected together by a symplastic stage.

The ultimate pleomorphist heresy was voiced by Wade and Manalang, when they stated that *Bacillus influenzae* (then thought to be the cause of influenza) could occasionally abandon its usual bacillary form, produce conidiophores, and grow as a "frank fungus" [8]. In the same year, the Swedish microbiologist Bergstrand journeyed all the way down this road by stating that bacteria are really *Fungi imperfecti* [9]. This view was also held by Mellon, who stated that:

bacteria in their fundamental biology are in reality fungi that have been telescoped down as it were, to a somewhat lower order, but this order is not so low as to preclude the preservation by the bacteria of the fundamental organisation characterising the fungi and higher plants [10].

Descriptions of pleomorphism in bacteria were often associated with bacteria isolated from tumors. There is an extensive literature implicating bacteria and other nonviral microorganisms in the etiology of cancer, many of which were said to be highly pleomorphic [11]. The best examples are provided by the work of Young, Clover and Gruner [12-14]. So impressed was the latter by the pleomorphic nature of his isolate that he named it *Cryptomyces pleomorpha*. Both Young and Clover provided illustrations showing complex life cycles, representing the passage of their cancer germs through a variety of stages including spores, bacilli, amorphous forms, and filamentous stages.

Not surprisingly, members of the monomorphic school had a field day criticising the apparently absurd claims made by the pleomorphists. The most common criticism was that the pleomorphists exhibited poor technique, their delusions obviously resulting from contamination. Secondly, the pleomorphists were said to have merely arranged whatever they saw, either contaminants or the products of ageing cells, into convenient life cycles. Winogradsky, perhaps not surprisingly, was severely critical of the pleomorphists, but nevertheless suggested that "*The observations may be correct*, but the interpretation given to the diverse forms observed cannot be taken seriously [15, my italics].

Perhaps the most impartial historical analyses of pleomorphism are given by Handly and Henrici, with the first chapter of the latter's book providing a particularly useful introduction to the history of pleomorphism [16, 17]. Although he was essentially critical of the concept of extreme pleomorphism, Henrici did not dismiss it as readily as many of his contemporaries. He stated that "bacteria do change their morphologic type and within very wide limits; and with this change may go at times important physiological modifications." Henrici particularly objected to the criticism that extreme pleomorphism always resulted from contamination; instead his opinion was that:

anyone who will patiently study with the microscope his own cultures which he knows to be pure can quickly confirm the general observation that rod forms may appear in cultures of cocci, spherical forms in cultures of bacilli lateral buds and branches and internal globular bodies.

Henrici finally came to essentially the same conclusion arrived at by Winogradsky, namely that "In undertaking a critical analysis of this work [of the pleomorphists] one cannot find fault *so much with the actual data* as to the logic followed in erecting the hypothesis [my italics].

The modern microbiologist, thoroughly schooled in monomorphism, can easily dismiss this historical literature as being absurd - merely the ramblings of some ancients who could not even avoid contaminating their cultures. While recognizing that some of the early studies were undoubtedly flawed, Wuerthele-Caspe et al., summed up the pleomorphist counterargument as follows: "the faults of the enthusiastic early workers were certainly no greater than the errors both of commission and omission made later on by some of the monomorphists whose views today dominate our textbooks" [18].

Young similarly defends the pleomorphist's case in a short yet comprehensive review which concludes the following quote:

Is all this [the evidence which he cites in support of pleomorphism] and a hundred and one similar observations by other careful workers merely a tissue of self-deceptions originating in an exuberant imagination and on faulty technique? Is it not rather one of those great facts that usher in a new era? [191].

While no such new era was ever ushered in, microbiologists will doubtless be surprised to discover that papers continue to appear in support of pleomorphism.

Recent Claims in Support of the Existence of Extreme Pleomorphism

Examples of pleomorphism continued to be reported with surprising regularity throughout the 1920s and 1930s. By 1940, however, opposition to the hegemony of the monomorphists was dead, if not yet buried. Textbooks on bacteriology nevertheless still gave token support to extreme pleomorphism even as late as the 1960s [20, 21]. During this period, work on L-forms appeared to substantiate some of the claims made by pleomorphists. Hieneberger-Noble, for example, suggested that L-forms correlated with the symplasm observed by Lohnis [22]. Bacterial conjugation, an idea that had been scoffed at by many monomorphists, was now taken seriously. Previously, Lohnis had been mocked when he had claimed that he, and numerous other workers including Potthoft, had observed conjugation tubes connecting two bacterial cells (see [23]).

Reports of the existence of limited pleomorphism continue to appear somewhat infrequently in the modern literature. Wood and Kelly, for example, recently showed that the morphology of a species of *Thiobacillus* varied in response to environmental conditions, while limited pleomorphism in *Bradyrhizobium* was reported by Reding and Leop to be induced by dicarboxylate [24, 25]. While claims for such limited pleomorphism offend no one, modern reports of extreme bacterial pleomorphism are likely to suffer derision, or more usually just be ignored.

The association between cancer etiology and bacteria continues to be the source of many of the claims made by modern pleomorphists. For example, an amazing series of papers linking extremely pleomorphic bacteria and cancer was reported in 1970 in a symposium in the *Annals of the New York Academy of Sciences*. The first of these papers, by Wuetherle Caspe-Livingston, reported the isolation of a specific type of highly pleomorphic microorganism found consistently in human and animal cancers [18]. Due to its remarkable pleomorphism, the organism was described as an "unclassified mystery," but was apparently capable of resembling micrococci, diphtheroids, bacilli, fungi, viruses, and host cell inclusions. Of particular interest was the reported appearance of an L-form, symplasm stage. The following quote from this paper could just as easily have come from the historical literature:

The virus-like bodies present in tumour and culture filtrates can evolve after one or more months into larger mycoplasma-like L forms, and thence to frankly bacterial rods and filaments. Polar or peritrichous flagella can develop under favourable conditions, and motile rods exhibiting a tumbling appearance. Under certain conditions unfavourable to the organisms, large glovoid bodies and still larger cysts from as well as spore forms develop. When conditions again become favourable, small bodies bud off from the chromatin ring lining the cyst, and filaments also may sprout from the rim. The small bodies, often acid fast, lengthen out into rods and filaments.

It should be noted however, that this work and the approach to cancer management which has been developed from it have come in for considerable criticism [26]. In particular it has been suggested that the so-called cancer germ involved is not new, but merely a strain of *Staphylococcus epidermidis*.

White also suggests that within cancerous cells exists "a non-septic, or non-virulent cell-wall deficient or conidial like micrococcus" [27]. Similarly intriguing recent papers reporting a complex life cycle in bacteria were written by Pease and Pease and Tallack [28, 29]. They state that for over a century there have been reports of a widespread, possibly universal, endoparasitism in humans caused by a bacterium capable of passing through a complex life cycle. Not surprisingly, this reported complexity has made this organism difficult to study and skeptics have yet to be convinced of its validity. The organism which Pease and Tallack consider to be a silent but potentially important pathogen was also reported to be associated with cancer by Alexander-Jackson and by Livingston and Alexander-Jackson [30, 31]. Incredibly, it has even been suggested that the Rous sarcoma virus is simple a stage in the life cycle of a bacterium. A number of workers have isolated cell-wall deficient bacteria from material containing the Rous sarcoma virus, as well as the Bittner virus and Shope's Papilloma virus [32]. Eleanor Alexander-Jackson even claims to have repeatedly grown cell-wall deficient bacteria from the blood of chickens infected with rous sarcoma [31]. Macomber apparently stated the obvious when he said that it goes against common sense to suppose that a virus can turn into a bacterium [32]. Instead, he suggested that it is more likely that viruses can be incorporated into cell-wall-less bacteria associated with the virus. He then emphasised the importance of clarifying the exact relationship between cancer-associated cell-wall deficient bacteria and the oncogenic retroviruses.

Pleomorphic cell-wall deficient bacteria have also been associated with rheumatoid arthritis. Mattman, for example, has claimed that a bacterium of this type, which apparently causes this disease in chickens, can revert in culture to *Propionibacterium acne* [33].

Conclusion

What can we make of all this? Most modern microbiologists, being monomorphists, would doubtless assume the examples of bacterial life cycles and extreme pleomorphism given here are merely the result of a mixture of wild speculation and contaminated cultures. (this was the view taken by Frobisher, who coined the word *oligomorphism* to describe the more readily acceptable examples, which clearly exist, of limited pleomorphism [20].) Yet most of the microbiologists who have reported examples of extreme pleomorphism went to considerable lengths to demonstrate the purity of their cultures.

It is also worth remembering that they often spent more time-much more than most modern microbiologists do-just looking at bacteria. Likewise, they were generally more practiced in the art of microscopy than are their modern counterparts. On the other hand, Holman and Carson showed that the work described by at least one researcher, who claimed to have demonstrated extreme bacterial pleomorphism, resulted from faulty bacteriological technique [32];.

Microbiologists of the past had no preconceived ideas about the nature of bacteria, and all possibilities were open to investigation. Of course, they lacked out technological sophistication - in particular, they knew nothing of the molecular approaches, which might be profitably used to study some of their apparently wild claims.

The literature on extreme pleomorphism remains intriguing, and some aspects of it may be worthy of reappraisal. By merely dismissing it, we may be ignoring something of fundamental importance. This is especially likely since examples of extreme variation in bacterial morphology continue to be linked with various diseases and cancer in animals and humans [33]. The use of molecular techniques should, however, help clarify any lingering uncertainties arising from the historical literature on extreme pleomorphism, although those certain of the phenomenon's validity would doubtless argue that their claims could be confirmed by simple, if thorough, microscopy. Perhaps it is now time to re-examine such claims with a non jaundiced eye.

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